A Fluorescence Based Assay for Chemically-Induced DNA Damage

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ABSTRACT

A simple, rapid and innovative assay to detect DNA damage is reported. This assay is based on repeated melting and annealing of double stranded (ds) DNA observed in real time using a fluorescence indicator dye. Chemically-induced damage to calf thymus DNA, caused by styrene oxide, is detected using this assay. The assay was characterized for styrene oxide-induced damage with respect to concentration, temperature and exposure times. At least 32 samples can be simultaneously analyzed using a 10 µL sample volume. The error in measurement was between 5 and 7%. The assay is reproducible and can be used to screen several chemicals known to cause DNA damage by several mechanisms.

BACKGROUND & INTRODUCTION

Rapid and inexpensive indicator assays that can be used to screen for the genotoxicity of contaminated environmental samples and which can be related to a biological target (e.g., DNA) could be of significant benefit to the exposure assessment process. A variety of short-term tests for genotoxicity/mutagenicity are currently used to determine the extent of environmental hazards resulting from polluted water and sediments. Despite the description of short term, however, many of these assays are expensive to run, require sophisticated technical expertise, and are not well suited to be adapted to field applications. The focus of this project is the characterization of rapid, sensitive and inexpensive assays for detection of damage to surrogate sequences of DNA caused by environmental pollutants and stressors. These methods are expected to provide the Agency with rapid, sensitive, and simple techniques that can be used among a panel of methods to determine the genotoxic potential of polluted samples.

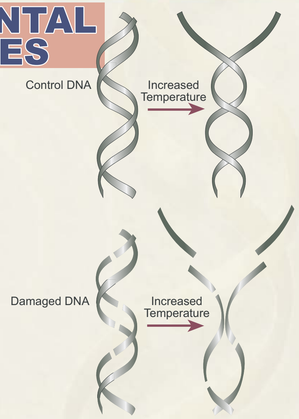
The damage to surrogate double stranded (ds) DNA (similar to genomic DNA) resulting from chemical interaction is a major concern from the environmental point of view. Styrene oxide forms adducts with the adenine and guanidine nucleotides within the DNA, resulting in DNA damage. Simple and inexpensive methods to detect DNA damage caused by chemicals such as styrene oxide can lead to the development of simple and inexpensive methods for screening a potentially wide range of genotoxic agents.

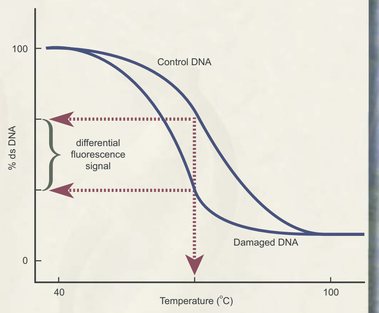
NOTICE

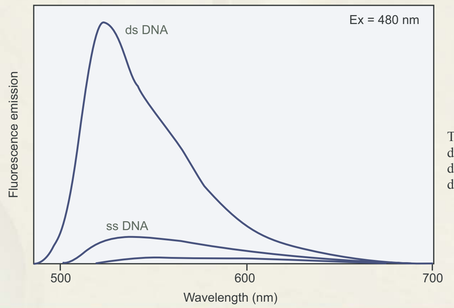
The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), funded this research and approved its presentation. The actual presentation has not been peer reviewed by EPA. Kumaran Ramanathan gratefully acknowledges a National Research Council Fellowship Program.

EXPERIMENTAL TECHNIQUES

The assay being developed for this project primarily detects single strand breaks in target DNA -- although this technique may also be sensitive to double strand breaks, adduct formation, and base losses. The concept for this assay is as follows: under certain conditions (i.e., high temperature or alkaline pH), double stranded DNA will unwind into single strands. Because temperature-induced unwinding (denaturation) occurs either more rapidly or under milder conditions if the DNA backbone has been broken along one of the strands, it can be used as an indicator of chemically-induced single strand breaks.







The degree of DNA denaturation is determined using the double strand sensitive dye PicoGreen. This dye indicator dramatically increases its fluorescence in the presence of double strand (as opposed to single strand) DNA.

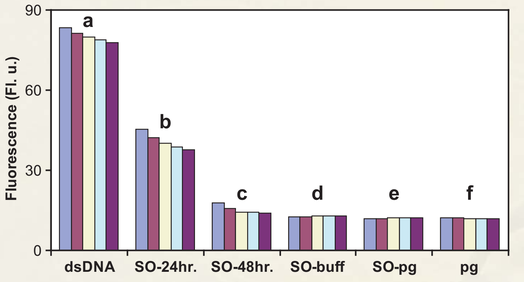
METHODS

dsDNA (40 µL at 100 ng/mL) was treated with either varying concentrations of neat styrene oxide (0.005 to 0.08 mole) or incubated for varying times using a fixed concentration. The optics within the instrument excited each capillary at 470 nm and the fluorescence emission at 530 nm was collected and stored in a data file. A temperature program was cycled between 30 and 99 °C for at least 5 cycles to study the melting and annealing behavior on damaged versus undamaged dsDNA.

RESULTS

Figure 1

Shows the effect of Styrene Oxide (SO) on melting and annealing behavior of ds calf thymus DNA. The fluorescence intensity of SO-treated (24 & 48 hr.) dsDNA (b, c) is lower than the intensity of untreated dsDNA (a). Controls are shown for SO in the absence of dsDNA (d), SO in the presence of PG (e) and PG in buffer (f). The indicator dye was PG-PicoGreen in Tris-HCl pH 7.4.



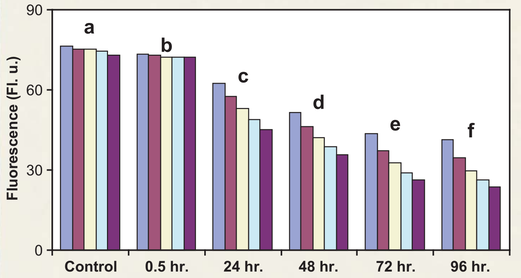
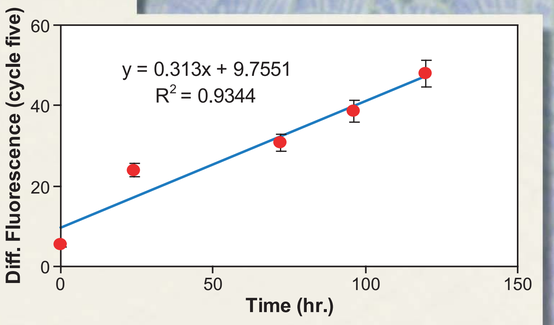


Figure 2

Shows the effect of exposure time of SO on the assay response. The average fluorescence intensity of control dsDNA (a) in the absence of SO remained unchanged during the course of the study. Changes in the fluorescence intensity for each melting/annealing cycle with time of incubation are shown in (b-f).

Figure 3

Shows a change in fluorescence (i.e., the loss of signal) at (cycle 5) between the untreated dsDNA and SO-treated dsDNA after various incubation times.



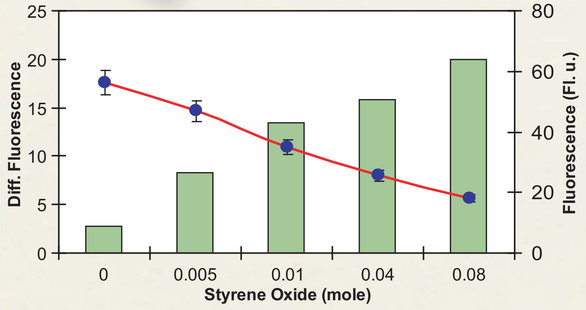


Figure 4

Shows the fluorescence difference (between cycle 1 and 5; i.e., the slope of the signal loss during successive melting/annealing cycles) for dsDNA treated with varying concentrations of SO between 0 and 0.08 moles. The line graph depicts the difference (at cycle 5) between the untreated and SO-treated dsDNA at each concentration.

SUMMARY & CONCLUSIONS

These studies demonstrate a fluorescence technique based on changes in melting/annealing behavior for measuring chemically-induced DNA damage. The fluorescence indicative of ds DNA damage decreased after exposure of the calf thymus to styrene oxide. The background fluorescence of the dye alone was unaffected by the damaging agent. Due to styrene oxide-induced DNA damage, the signal decreased at the beginning of the initial melting/annealing cycle. The signal also decreased at the beginning of each successive melting/annealing cycle. This result suggested that the damaged ds DNA annealed less efficiently than the undamaged DNA.

The herein reported screening assay showed the following characteristics in measuring styrene oxide-induced DNA damage:

- simple
- rapid (15 min assay time)
- high-throughput (32 samples / assay)
- low volume (10 µL)
- requires small amounts of DNA (1 ng/assay)